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GERANYLHYDROQUINONE: A CONTACT ALLERGEN FROM TRICHOMES OF *PHACELIA CRENULATA*

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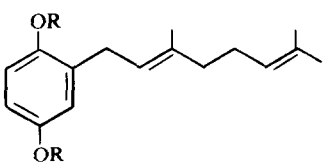
Key Word Index—*Phacelia crenulata*; Hydrophyllaceae; trichomes; contact dermatitis; geranylhydroquinone.

INTRODUCTION

Several species of *Phacelia* (Hydrophyllaceae) are known to cause an allergic contact dermatitis of which the greatest number of cases reported are from *P. crenulata* [1, 2]. The allergenic species of *Phacelia* have stalked glandular trichomes each of which produces a spherical drop of oil on the capitate head. The report by Munz [1] suggested that these trichome oils were responsible for the dermatitis, and so we directed our attention to the trichomes in looking for an allergenic compound. We have begun our study with *Phacelia crenulata* var. *funerea* J. Voss and have found that the main constituent of the trichome oil is geranylhydroquinone (**1**). This we found to be a potent skin sensitizer for guinea pigs, which have proven to be fairly reliable indicators of allergenicity against human skin [3]. This is the first report of geranylhydroquinone from plants. It has been isolated from the tunicate *Aplidium* [4], and it has been synthesized as a drug which is reported to have some value as a radioprotective and anti-cancer protective agent [5, 6].



1 R = H
2 R = COMe

RESULTS AND DISCUSSION

A crude extract from acetone plant washings was chromatographed on a Si gel column and the fractions were assayed for allergenicity on guinea pigs previously sensitized to the crude extract. The allergenic component was the major constituent of the trichome exudate, which we determined was geranylhydroquinone. We established that this was in fact a constituent of the glandular trichomes by plucking several trichomes from the surface of the plant with fine forceps, eluting them in acetone and developing on TLC.

The UV max was 295 nm. The MS of the acetylated derivative gave a molecular ion at *m/e* 330 with a base peak the dihydroxytropylium ion at *m/e* 123. Other major ion peaks were at *m/e* 287, 246, 177, 161, 69, 43 and 41 consistent with the fragmentation expected for structure **2**. The ^{1}H NMR spectrum in $CDCl_3$ produced chemical shifts at δ 1.58 (3H), 1.68 (6H), 2.05 (4H), 3.27 (2H, *d*, J = 7 Hz), 5.16 (1H, 5.30 (1H), 5.87 (2H) and 6.63 (3H). Geranylhydroquinone was synthesized by condensation of geraniol and hydroquinone in 1% oxalic acid. The allergenic constituent of *P. crenulata* had identical UV, ^{1}H NMR, and IR spectra as the synthesized compound.

Configuration of the double bond at C-2, C-3 was established as *trans*. Inouye *et al.* [7] observed that acetylation of nerylhydroquinone caused a downfield shift of the ^{1}H NMR resonance of the C-4 methyl, while acetylation of geranylhydroquinone produced an upfield shift. Acetylation of the *Phacelia* compound produced an upfield shift of the C-4 methyl of 3 ppm (δ 1.68 to 1.65), which was identical to that of the synthesized compound and its acetylated derivative.

The minimum dose of geranylhydroquinone isolated from *Phacelia* required to produce an irritant skin reaction on non-sensitized guinea pigs was 500 μg (applied as 5 μl of acetone solution in an 8 mm dia circle). An allergic skin reaction was produced on all of eight sensitized animals with 60 μg of sample and on three of eight animals with 20 μg.

A screening of other species of *Phacelia* by TLC has indicated that *P. minor*, *P. parryi*, *P. viscida* and *P. companularia* also have geranylhydroquinone, while *P. distans*, *P. cicutaria*, *P. fremontii* and *P. minutiflora* do not. The former species have all been reported to be allergenic and cross-reactive [1]. *P. distans* has also been implicated as being allergenic, but it apparently does not cross-react with other allergenic species of *Phacelia* [1].

EXPERIMENTAL

Extraction and isolation. Phacelia crenulata var. *funerea* was collected 30 m east of Barstow, Calif. in April, 1978. Voucher specimen is on deposit at the Museum of Systematic Biology, University of California, Irvine. Fresh whole plants (10 kg) were washed 5–10 sec in Me_2CO; the extract was filtered and concd to yield 25 g of crude oil. This was chromatographed on a Si gel column eluted with $CHCl_3$. Pure samples for analysis were isolated on a Waters Associates HPLC using a μ-Porosil column eluted with $CHCl_3$–*iso*PrOH–HOAc (100:1:0.4)

Synthesis of geranylhydroquinone. A mixture of 7.8 g geraniol, 5.5 g hydroquinone and 25 ml 1% aq. oxalic acid was heated in a H_2O bath at 80–90° with stirring for 1 hr. The mixture was extracted with C_6H_6 and chromatographed on a Si gel column with $CHCl_3$. Yield was 20%.

Guinea pig assay for allergenicity. Hartley strain guinea pigs 2–4 months old of mixed sex were sensitized by intradermal injection of trichome extract in Freund's complete adjuvant (FCA). FCA was prepared as described in ref. [8]. 320 mg of plant extract was mixed with FCA to a vol. of 5 ml. Each of 8 animals received a total of three 0.1 ml injections intradermally in the interscapular area; one injection was given every other day. A control group of 7 animals received the same

injections of FCA but without plant extract. Two weeks after the last injection, the animals were depilated and challenged with serial dilutions in Me_2CO of fractions or pure compounds obtained from chromatography. A 5 μl sample was applied in an 8 mm dia circle by a syringe pipetor. The animals were checked for skin reactions at 24, 48 and 72 hr.

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PLAGIOCHILIDE FROM THE LIVERWORT, *PLAGIOCHILA ASPLENIOIDES*

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Key Word Index—*Plagiochila asplenioides*; Jungermanniales; Hepaticae; plagiochilide; sesquiterpene; ^{1}H NMR; ^{13}C NMR.

A $CHCl_3$ extract of *Plagiochila asplenioides* (L.) Dum. collected in Switzerland afforded a secoaromadendrane-type sesquiterpenoid for which we propose structure **1** on the basis of spectral data. Asakawa *et al.* recently reported the sesquiterpenoid plagiochilide (**1**) from *Plagiochila yokogurensis* [1]. That the compound from *P. asplenioides* corresponds to **1** was confirmed by MS (obs. *m/e* 232.146, calc. 232.146) and by comparison of IR and ^{1}H NMR with those of an authentic specimen. The ^{13}C NMR data also confirm the structure (Fig. 1).

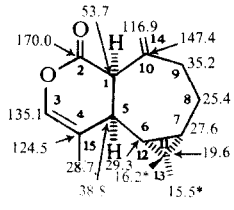

Fig. 1. Plagiochilide (**1**) and its ^{13}C NMR data (ppm, TMS, $CDCl_3$. * Assignment may be changed.

Asakawa *et al.* also recently isolated four novel seco-aromadendrane-type sesquiterpene hemiacetals (plagiochilines C, D, E and F) from a collection of *P. asplenioides* from France [2]; however, they did not detect plagiochilide. The systematic implications of the chemical differences between the Swiss and French specimens of *P. asplenioides* will require studies of collections from other areas.

EXPERIMENTAL

Mps are uncorr. ^{1}H and ^{13}C NMR were measured on HA-100 (Varian) and WH-90 (Bruker), respectively. Si gel 60 (70–230 mesh, Merck) and Si gel 60 GF 254 (Merck) were used for column, TLC and PLC (1.0 mm). Petroleum ether (PE) refers to 30–60° bp range.

Extraction and separation. Plagiochila asplenioides (204 g air-dried material) collected in August, 1977 near Brienz, Bernese Alps, Switzerland (voucher was deposited at the Herbarium of the Fachrichtung Botanik, Universität des Saarlandes, Saarbrücken) was extracted with $CHCl_3$. The solvent was removed *in vacuo* to give 4 g gummy dark syrup. The syrup was chromatographed on a Si gel column (80 g) using a PE–Et_2O gradient elution system. The fraction which eluted with 75% PE and 25% Et_2O was purified further on PLC (PE–Et_2O, 3:1 and 2:1)